ION REGULATION IN TETRAHYMENA [1]

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Fresh water animals maintain their cells hyperosmotic to their environment (Prosser *et al.*, 1950). In higher animals this is accomplished by specialized organs or tissues (*e.g.* the vertebrate kidney, frog skin, and the anal papillae of dipteran larvae) which adjust the osmotic level of body fluids to a level isosmotic with the cells.

Lower invertebrates (protozoa, sponges and coelenterates) have no osmotically regulated body fluids. Therefore all cells in fresh-water representatives of these groups have the problem of continuous water influx, and must have osmoregulatory ability (Kitching, 1954).

A high potassium content relative to their medium is characteristic of all living cells that have been investigated. Most cells are richer in potassium than sodium, and there is often less sodium in cells than in the medium. Evidence has been offered that in such fresh-water animals as *Hydra* and *Spirostomum*, inorganic ions are readily available for exchange with the environment (Lilly, 1955; Carter, 1957). Table I lists potassium and sodium levels in several fresh-water invertebrates. Therefore regulation of body volume in such forms involves regulation of ions as well as water.

This paper reports an investigation of ionic regulation in *Tetrahymena pyriformis*, a fresh-water ciliate. A remarkable ability to maintain a high potassium concentration as well as a lower sodium concentration in very dilute medium was found. Evidence for a sodium extrusion mechanism was also found. These findings will be discussed in terms of a model system for ion regulation in *Tetrahymena*, and in terms of relevance to similar problems in other animals.

Methods and Materials

Tetrahymena pyriformis, strain W, was grown axenically in 2% proteose-peptone medium (hereafter called normal medium), the ion content of which is indicated in Table II. One-liter Roux culture bottles containing 500 ml. of medium were innoculated with 5 ml. of a culture in log phase of growth. After four days' growth at 22–25° C., the cells were concentrated approximately ten-fold by gentle centrifugation. After experimental treatment, which involved either dilution of cell suspension in normal medium with distilled water or increasing medium

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TABLE I

K and Na (or osmolar) concentrations of some fresh water invertebrates. (See Willmer (1958) for a table of osmolar concentrations of some protozoans determined by a variety of methods.)

Organism	Concentration	Method of determination	Literature source
Acanthamoeba (distilled water washed)	K—26.9 (meq./l. cells) Na—14.3	elemental analysis	Klein (1959)
Spirostomum	K—7 (meq./l. cell water) Na—1	equilibration with isotopic tracer	Carter (1957)
Tetrahymena (in 2% proteose-peptone)	K—31.7 (meq./l. cells) Na—12.7	elemental analysis	Dunham and Child (present report)
Spongilla (summer)	osmolarity equivalent to 27 meq. NaCl/l. cell water	vapor pressure determination	Zeuthen (1939)
Pelmatohydra (whole animal)	K—14.4 (meq./l. cell water) Na—2.7	equilibration with isotopic tracer	Lilly (1955)
Tubifex (whole animal)	K—27.0 (meq./kg. wet Na—23.4 weight)	elemental analysis	Dunham (unpublished)
Anodonta (muscle)	K—10.6 (meq./kg. wet Na—5.2 weight)	elemental analysis	Hayes and Pelluet (1947)

concentration by adding NaCl or KCl, cell suspensions were reconcentrated when necessary so that 10 ml. gave a packed cell volume of about 0.2 ml., as determined by centrifugation at a relative centrifugal force of 1600 to constant volume (10 minutes in 10-ml. Kolmer tubes).

Dry weight of cells was determined by drying to constant weight at 60° C. Cell counts were made in a hemocytometer.

Cells were extracted for ion analyses by suspending them in dilute acetic acid (1 drop glacial acetic acid in 10 ml. water), heating near boiling for 5 minutes and allowing them to stand for one hour. K and Na analyses were made with a Coleman model 21 flame photometer. Preliminary Cl analyses were made with an Aminco-Cotlove chloride titrator. Analysis of nitric acid digests of residues after extraction indicated that more than 98% of intracellular K, Na and Cl was extracted. Intracellular cation concentrations are expressed in meq./l. cells, after appropriate correction for extracellular space as determined by use of radioactive iodinated serum albumin (Risa) added immediately prior to centrifugation. Total exchangeability and kinetics of intracellular K and Na were determined using trace

TABLE II

K and Na concentrations of Tetrahymena *in 2% proteose-peptone (normal) medium. K, Na, and Cl concentrations of normal medium. Standard errors and number of determinations are given.*

Cells	meq./l. cells
K	31.65±0.43 (44)
Na	12.68±1.35 (44)
Medium	***mM***
K	4.75±0.13 (60)
Na	36.5±0.13 (70)
Cl	28.7±1.02 (6)

amounts of the appropriate radioisotope, K^{42} or Na^{24}, obtained from Oak Ridge National Laboratories as chlorides in HCl solution, and neutralized with NaOH before use. Counts per minute of wet samples were determined with an end-window counter or a NaI-Tl crystal scintillation well counter. Per cent exchanges of intracellular K and Na were calculated from the specific activities of the cells and of the medium.

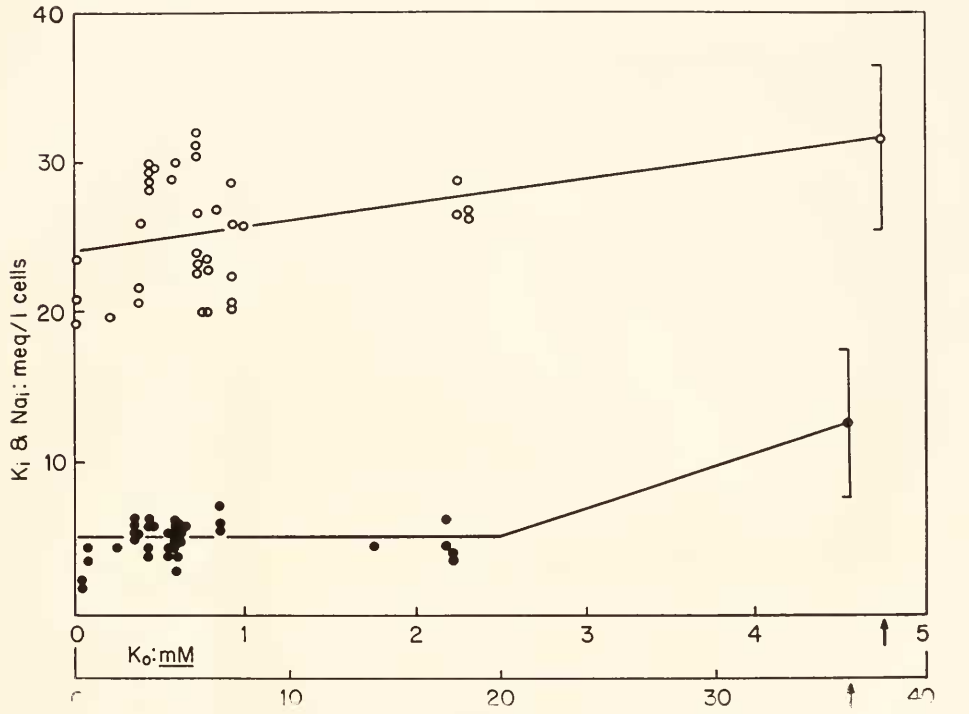

FIGURE 1. K and Na content of *Tetrahymena* in normal and diluted media. Ordinate: cellular concentrations of K and Na (meq./l. cells); abscissas: concentrations of K and Na in the medium (*mM*). Open circles: K_i; solid circles: Na_i. Points for K_i and Na_i in normal medium (see arrows) are averages of 44 determinations; vertical lines delimit the total range of the determinations in normal medium.

RESULTS

Table II shows the K and Na content of cells in normal medium, and the K, Na and Cl content of normal medium. The volume and weight of an average cell in normal medium were 1.83×10^{-5} μl. and 1.97×10^{-2} μg., respectively, as determined from cell counts and packed cell volumes (corrected for Risa space). Dry weight of cells in normal medium was determined to be 19.4% of wet weight, so cells are 80.6% water. The percentage of Risa space in a volume of packed cells in normal medium was 15% (eight determinations ranging from 14% to 18%, SE = 0.57). This value was not significantly different for cells equilibrated with medium diluted eight-fold.

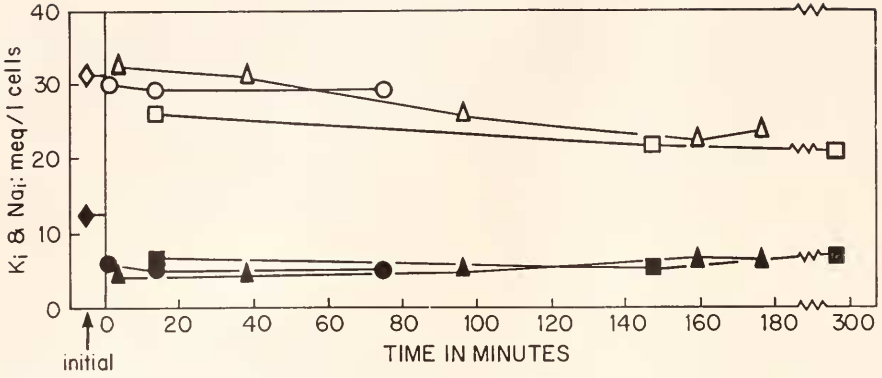

FIGURE 2. Changes in cellular K and Na in *Tetrahymena* after dilution of normal medium. Ordinate: cellular K and Na concentrations (meq./l. cells); abscissa: time (minutes). Open symbols: K_i; solid symbols: Na_i. Initial K_i and Na_i (diamonds) are averages of values in normal medium. Dilutions were made at zero time. The extents of the dilutions of normal medium in the three experiments were as follows: triangles, 6-fold dilution; circles, 8-fold dilution; squares, 13-fold dilution.

In order to demonstrate how intracellular K and Na are maintained over a range of medium concentrations, cells were allowed to equilibrate for at least 30 minutes in various dilutions of normal medium from two-fold to over 100-fold, the extreme dilution involving several distilled water washes. Figure 1 shows

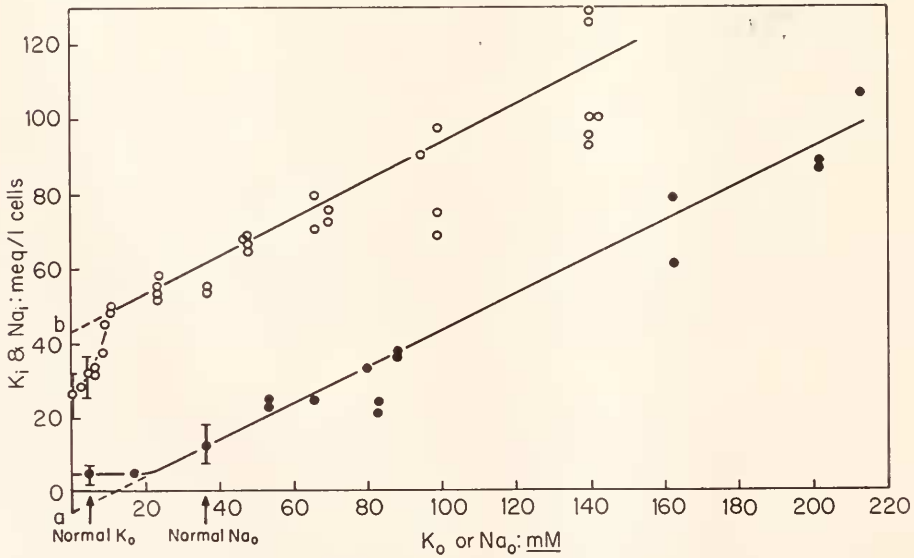

FIGURE 3. K and Na content of *Tetrahymena* in media concentrated with KCl or NaCl. Ordinate: cellular concentrations of K and Na (meq./l. cells); abscissas: concentrations of K and Na in the media. Open circles: K_i; solid circles: Na_i. *a* and *b*: ordinate intercepts of the linearly increasing portions of the Na and K curves, respectively. Points for K_i and Na_i in normal and diluted media are taken from Figure 1.

final intracellular concentrations of K and Na (K_i and Na_i) plotted against medium concentration (K_o and Na_o). K_i and Na_i are quite constant over the range of medium dilutions investigated: average $K_i = 25.4$ meq./l. cells and average $Na_i = 5.0$ meq./l. cells; $K_i/Na_i = 5.1$. Figure 2 shows the results of three experiments in which changes of K_i and Na_i were followed after six-fold and greater dilutions of normal medium. Changes in Na_i take place within the first 30 seconds, after which Na_i is constant. K_i decreases very slowly after medium dilution, so it is difficult to assign an equilibrium level. Therefore it was arbitrarily decided that K_i values in cells in dilute medium more than 30 minutes would be reported with the reservation that time for equilibration may involve a matter of days. (In one experiment, K_i in cells in medium diluted 20-fold for two days was about half normal K_i.) Rates of decrease of K_i in cells in dilute medium were never greater than 10% per hour, and generally were much slower.

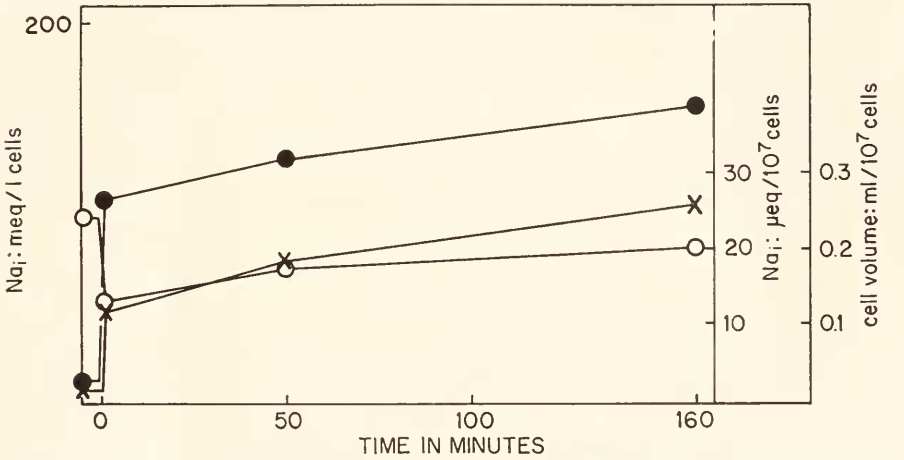

FIGURE 4. Changes in volume and Na content of *Tetrahymena* after increasing the NaCl concentration of normal medium. Ordinates: cell volume (open circles); Na content per unit number of cells (crosses); Na content per unit volume of cells (solid circles). Abscissa: time (minutes). NaCl concentration of normal medium was increased by 176 *mM* at zero time. K_i per unit number of cells at 160 minutes was not significantly different from initial K_i.

Cells were equilibrated for 30–120 minutes in media made more concentrated than normal in either K or Na (added as chlorides). Figure 3 shows K_i and Na_i values from these experiments plotted against K_o and Na_o, respectively. Both curves are linear above certain medium cation concentrations, with values of 0.51 for the slope of K_i/K_o above 11 *mM* K_o and 0.48 for the slope of Na_i/Na_o above 20 *mM* Na_o. Below 20 *mM* Na_o, Na_i is constant at 5.0 meq./l., whereas below 11 *mM* K_o, the K curve is roughly sigmoid.

The kinetics of net influx of cation and the concomitant water movements were investigated by subjecting cells to sudden large increases in K_o or Na_o. Changes in packed cell volume, cation concentration per unit volume of cells, and cation concentration per unit number of cells were followed. Figures 4 and 5 show the results of subjecting cells to increases above normal of 176 *mM* NaCl and 137 *mM* KCl, respectively. In both cases there is a large initial influx of the

elevated cation (shown by increase in μeq./10^7 cells) and large efflux of water (45% cell shrinkage in each case) in the first 1.5 minutes. K_i per cell increases initially about 1.75 times, and subsequently increases slowly to 4.5 times the initial level by 200 minutes, accompanied by water re-entry. Na_i per cell increases initially to 6 times initial level, and subsequently slowly increases to 13.7 times initial level at 160 minutes, with water re-entry. So equilibration in both cases is fast, but much faster in high Na medium. When Na_i increased, final K_i per cell was not significantly different from the initial value, and likewise for Na_i per cell after 200 minutes when K_i increased. This lack of reciprocal changes means that increases in K_i and Na_i are accompanied by proportionate increases in

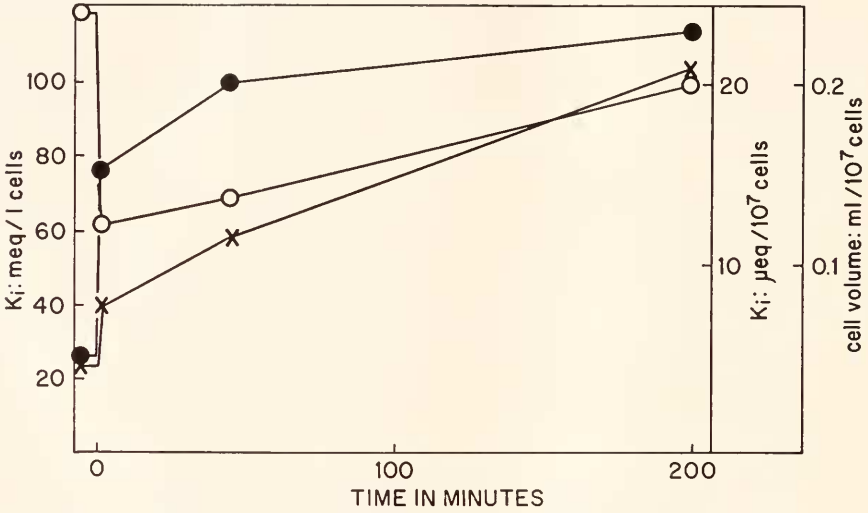

FIGURE 5. Changes in cell volume and K content of *Tetrahymena* after increasing KCl concentration of normal medium. Ordinates: cell volume (open circles); K content per unit number of cells (crosses); K content per unit volume of cells, solid circles. Abscissa: time (minutes). KCl concentration of normal medium was increased by 137 *mM* at zero time. Na_i per unit number of cells at 200 minutes was not significantly different from initial Na_i.

some anion, or decreases in some other cation, if electroneutrality is preserved within the cells. (In a few experiments in which both K_o and Na_o were increased, both K_i and Na_i increased in the same way as when studied singly, as described.)

Preliminary analyses of Cl content of cells show that Cl does not balance increases in K_i or Na_i. Figure 6 shows Cl_i values in cells in normal, dilute, and high NaCl and KCl media. Cl_i is quite constant in media ranging from a 2-fold dilution (14 *mM* Cl_o) up to 123 *mM* Cl_o (99 *mM* K_o) and 73 *mM* Cl_o (83.5 *mM* Na_o): for 18 determinations, Cl_i averaged 6.4 meq./l. cells, ranging from 0 to 13.2 meq./l. One set of determinations at 150 *mM* Cl_o (163 *mM* Na_o) showed Cl_i as high as 37 meq./l., but Na_i was 78.5 meq./l. in this case. For this one deviant value, only one-half of Na_i could be balanced by Cl, all other determinations showing no relationship at all between Cl_i and K_i or Na_i.

Preliminary experiments were done on the washout of high K or high Na content of cells transferred to normal medium after one hour's equilibration in

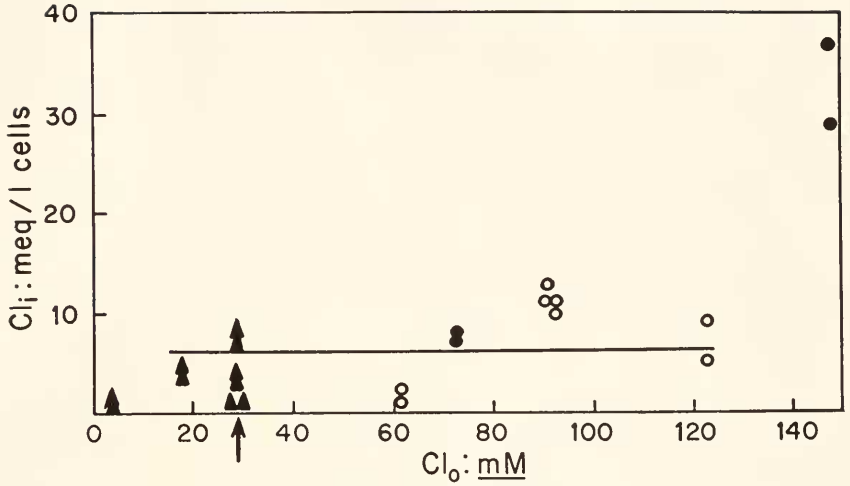

FIGURE 6. Cl content of *Tetrahymena* in normal, diluted, and high NaCl and KCl media. Ordinate: cellular concentrations of Cl (meq./l. cells); abscissa: Cl concentration of the media. Triangles: Cl_i in normal and diluted media; open circles: Cl_i in high KCl media; solid circles: Cl_i in high NaCl media. Arrow indicates Cl concentration of normal medium. Horizontal line indicates average of all Cl_i values in medium Cl concentrates ranging from 17 *mM* to 123 *mM*.

high K or Na medium. Cells were equilibrated in normal medium with the KCl concentration increased by 65 *mM*. Ten minutes after washing with normal medium, K_i had decreased from 64 meq./l. to 57 meq./l., and after 45 minutes to 48 meq./l. Initial K_i was 33 meq./l. Therefore a portion of elevated cellular K washed out much more slowly than it can be increased. This observation is consistent with the slow decrease of K_i from cells after dilution of normal medium. Cells were also equilibrated for one hour in normal medium plus 170 *mM* NaCl, then washed with normal medium. Ten minutes after washing, Na_i was only slightly above normal Na_i, indicating that Na_i is easily washed out of cells, as a portion of Na_i is after dilution of normal medium.

TABLE III

Kinetics and extent of exchange of cellular K and Na with normal and diluted media containing trace amounts of K^{42} or Na^{24}. Each horizontal row of data represents at least two experiments with replicate determinations.

	Medium conc. (*mM*)	Total cell conc. (meq./l. cells)	Time for maximum exchange (minutes)	Time for ½ maximum exchange (minutes)	Exchangeable cell content (meq./l. cells)	Unexchangeable cell content (meq./l. cells)	Exchangeable fraction
K, normal medium	4.75	31.6	180	30	29.2	2.4	92.5%
K, diluted medium	0.83	27.4	180	30	25.0	2.4	91.0%
Na, normal medium	36.5	12.7	120	<1	11.2	1.5	88%
Na, diluted medium	3.0	5.5	120	3	3.25	2.25	59%

Table III summarizes the results obtained when cells were exposed for 5 or more hours to normal and diluted media containing trace amounts of K^{42} or Na^{24}. Cellular K and Na are largely available for exchange with medium K and Na, and exchange is rapid, particularly the exchange of Na. However, small amounts of both K and Na do not exchange in a 5-hour period, although the exchange reaches a maximum level by three hours for K_i and two hours for Na_i. The amounts of unexchangeable K_i and Na_i do not change significantly with medium dilution.

Discussion

That *Tetrahymena* is capable of maintaining a high cellular K content relative to the K content of normal and diluted media is evident from Figure 1 and Table II. Ratios of K_i/K_o are of the order of 100 and higher in very dilute medium. *Tetrahymena* also retains a small amount of Na in very dilute medium.

Sizable portions of cellular K and Na are exchangeable with the medium, as shown in Table III. Therefore K and Na are not retained in the cells by an impermeable membrane. Table III also shows, however, that small and constant amounts of both K and Na are unexchangeable. Since net changes are also evident, exchange diffusion cannot be responsible for the ready exchange of isotopes.

A system of internal binding sites with specific affinity for K is suggested to explain active K maintenance by *Tetrahymena*. Na may be retained by a similar mechanism. In addition, a Na extrusion mechanism is proposed.

Na in *Tetrahymena* is best explained in terms of a formal model involving compartmentalization of Na_i. Two components of Na_i are constant, *i.e.* do not vary with Na_o. One, 1.9 meq./l., is unexchangeable with the medium. The second, 3.1 meq./l., is held constant, but is rapidly exchangeable. Forty-eight per cent of cell volume is free "Na space," and is available to a mobile Na component which is freely diffusible, and proportionate to Na_o. However, this mobile component is maintained 20 meq./l. of water less than Na_o by Na extrusion. Below Na_o there is no mobile Na component and Na_i is constant at 5 meq./l., but at 20 *mM* Na_o the Na extrusion mechanism is operating maximally and mobile Na_i begins increasing with Na_o. Since the other two Na components are constant, total Na_i increase is linear and represents only the mobile component. There are no net exchanges between any of the compartments. These compartments can be visualized as physiological entities only, since no morphological significance can be attached to them. This model is suggested by the following points of evidence:

(1) Na extrusion is indicated first, by the constant level of Na_i up to 20 *mM* Na_o, and second, by the negative intercept on the ordinate axis of the linearly increasing portion of the Na_i/Na_o curve (indicated in Figure 3). (Permeability of the cells to Na precludes passive exclusion of Na.)

(2) The mobility of Na_i above the constant 5 meq./l. is apparent from the rapid equilibration of cells with high Na medium, and the rapid washout of Na from cells both when normal medium is diluted and when high Na cells are washed in normal medium.

(3) The linearity of the Na_i/Na_o curve above 20 *mM* Na_o and the mobility of Na_i above 5 meq./l. allow one to conclude that the slope of the Na curve, 0.48, represents the fraction of cell volume occupied by the mobile Na component, or "Na space."

(4) The magnitude of the gradient effected by maximum Na extrusion is proportional to the magnitude of the negative intercept (about 5 meq./l. cells) corrected for the constant amount of retained Na_i (5 meq./l. cells), or 10 meq./l. cells. Since the Na space is 48%, the difference in Na concentration between medium and cell water is $10/0.48 = 20.8$ meq./l. water. This value should and does correspond closely to the medium Na concentration at which the Na extrusion mechanism becomes saturated. This saturation concentration, the "threshold" of Na_i increase, is analogous to the threshold of glucose excretion in renal tubules at the concentration of maximum glucose reabsorption (Shannon and Fisher, 1938), which was also interpreted as representing saturation of an accumulating mechanism.

(5) The evidence for the unexchangeable and exchangeable but constant components of cellular Na has already been presented.

The nature of the preservation of electroneutrality in the cells upon Na or K entry is not at all clear. There is definitely no reciprocal relationship between Na and K. There is little intracellular Cl, even upon large increases in the medium of either NaCl or KCl. These results also obviate explaining active retention of K in terms of a Donnan equilibrium resulting from Na extrusion, unless Cl is also specifically excluded from the cells, which seems unlikely at present. If a Donnan situation obtained, the relationship $K_i/K_o = Cl_o/Cl_i$ would be expected to hold in any medium, and it obviously does not. With increasing medium KCl, K_i/K_o decreases to less than 1, while Cl_o/Cl_i increases to as high as 22. The nature of the Cl exclusion is suggested here to be electrostatic rather than a matter of specificity or impermeability. Possibly cellular K and Na are associated with fixed anionic groups more or less strongly, depending on the ion and the medium concentration of the ion. Steinbach (1947) suggested that K is always associated with organic components which occupy spaces unavailable to Cl. This still does not explain the preservation of electroneutrality. This problem is under investigation.

The K_i/K_o gradients in normal and diluted media are definite evidence for specific K retention. The rapid and nearly complete exchange of K_i and the rapid net increase in K_i with K_o make membrane impermeability or any membrane involvement unlikely explanations for K retention.

In media with K_o higher than normal, the slope of the K_i/K_o curve is steep up to 11 *mM* K_o, above which K_i increases less sharply with K_o, and in a linear fashion. The slope of this portion of the curve is 0.51, close to the slope of the Na_i/Na_o curve (0.48), suggesting that this increasing K_i is occupying a cellular space identical with the "Na space."

However, a portion of this increased cellular K does not readily wash out of the cells, and is not nearly as mobile as high cellular Na. Cells equilibrate less rapidly with high K medium than with high Na medium. Increased K_i is not accompanied by reciprocal Na changes or by Cl_i increase, as noted above. These considerations suggest that the increase in K_i involves association with previously "empty" K binding sites in the "Na space." (Steinbach (1940) reported K increase without Na decrease or Cl increase in *Phascolosoma* muscle. He suggested (1947) that vertebrate skeletal muscle behaves as though there were a limited number of groups capable of binding K which are normally saturated, whereas heart and invertebrate muscle are normally not saturated with K.) The slower rise of K_i

with K_o greater than 11 *mM* and the linearity of this rise suggest that the actively maintained, or bound, K is at a maximum above 11 *mM* K_o. Then the intercept of the linear portion of the K_i/K_o curve at the ordinate axis, 43 meq./l. (shown in Figure 3), should be the level of maximum actively maintained K. In cells equilibrated in high K medium, and washed in normal medium, K_i fell to 48 meq./l. after 45 minutes, a concentration comparable to the ordinate intercept of the linear K_i/K_o. This constitutes additional evidence for the maximum saturation of bound K. Initially it would appear that K maintenance in *Tetrahymena,* because of the sigmoid shape of the K_i/K_o curve below 11 *mM* K_o, does not fit elementary Langmuir adsorption theory (applicable to Michaelis enzyme kinetics), which it should if a system of binding sites with a saturation level is invoked. However, it is likely that this is not the true shape of the curve. The K washout experiment described above shows that K_i, although it can be rapidly increased, can be only slowly washed out. Since the initial K_o in the experiments involving changing K_o was always that of normal medium, an inflection in the K_i/K_o curve is expected at the K_o of normal medium, and it is observed (see Figure 2). Therefore the explanation of the sigmoid shape of the curve lies not in the nature of the K retention mechanism, but in the experimental procedure. Then the real relationship between actively maintained K and K_o should fit a Langmuir adsorption isotherm, and a binding sites mechanism for K retention is consistent with the data.

No comparable obscuring factor exists in the case of Na_i since Na apparently washes in and out of cells with equal facility in the range of medium concentrations investigated. The Na retention system is apparently saturated at a very low Na_o.

The data suggest compartmentalization of K as well as Na: unexchangeable K (2.4 meq./l.), exchangeable but bound K (maximum about 43 meq./l.), and freely diffusing K, with no threshold K_o. (See Cowie, Roberts and Roberts, 1949, for a discussion of compartmentalization of K in *E. coli.*)

The hypothesized ion regulatory machinery of *Tetrahymena,* shown to be consistent with the data, consists, first, of a system of internal binding sites which specifically accumulate and retain K; second, a system for retention of a constant, low level of Na, and third, a Na extrusion mechanism. Cl plays little role in ion balance in *Tetrahymena.* Cellular K and Na are separable into three components: unexchangeable, exchangeable but bound, and mobile components.

The Na extrusion mechanism may facilitate water removal, and therefore may be associated with the contractile vacuole. The water economy of *Tetrahymena* may be analogous to that of other fresh-water animals, in that *Tetrahymena* may not be capable of secreting pure water, but water removal may be facilitated by ion secretion (cf. Prosser *et al.,* 1950).

The relationship of Na retention to Na extrusion and/or K retention, cannot be decided from the data. Retained Na may represent lack of complete specificity of the K binding sites, but in this case a reciprocal relationship between actively retained K and Na would be expected. The retained Na might be a reservoir necessary for vacuolar function. This possibility is consistent with the rapid Na turnover indicated by rapid exchange, but cannot be easily resolved with the constancy of the retained Na relative to Na_o. A third possibility is a specific protoplasmic requirement for a low, constant Na level, for which there is no

evidence here and little precedent. (*Hydra* (Lenhoff and Bovaird, 1960) and *Chilomonas* (Pace, 1941) have possible specific Na requirements.)

The specificity of K retention in *Tetrahymena* indicates a protoplasmic K requirement. (Kidder *et al.* (1951) demonstrated a nutritional requirement for K in *Tetrahymena.*) The similarity of levels of K in *Tetrahymena* and the other animals listed in Table I suggests a minimum requisite protoplasmic level of K. It is often held that high cellular K is only a reflection of a Donnan equilibrium resulting from Na extrusion (see Hodgkin, 1951). Carter (1957) attributes K maintenance in *Spirostomum* to Na exclusion. This has been shown here not to be so in *Tetrahymena,* and Robertson (1957) has shown that in a number of animals, including some marine invertebrates, a portion of cellular K cannot be accounted for by a Donnan equilibrium, but must be due to specific K retention. Steinbach (1947) suggests that cellular K is regulated relative to a constant protoplasmic composition rather than to serve an osmoregulatory function. No doubt in marine animals and vertebrates, with their relatively high ionic content, some cellular K is held non-specifically to preserve electroneutrality. Cellular K levels vary considerably, particularly among fresh-water animals, and probably reflect the ability to regulate body fluids, and to an extent the activity of the animal. Therefore in vertebrate cells, there may be a K component reflecting a Donnan equilibrium plus a component serving a specific protoplasmic role. Since evolution of basic cellular mechanisms is generally conservative, a similarity between K retention mechanisms in *Tetrahymena* and other animals is an attractive possibility. *Tetrahymena* affords a system for studying this mechanism without high ion concentrations and large Donnan effects, which would obscure specific K retention in other animals.

Akita (1941) reported data on Na, K and Cl contents of *Paramecium* which were comparable to the data presented above on *Tetrahymena.*

Summary

1. The K and Na content of *Tetrahymena pyriformis* has been determined, and the mechanisms of ionic regulation were investigated.

2. The main findings were: K and a small amount of Na are maintained in very dilute medium. Cellular K and Na are readily exchangeable with K and Na of the medium. However, small, constant amounts of each are unexchangeable. Cells rapidly equilibrate with media high in K or Na. High K washes out of cells slowly, whereas Na enters and washes out of cells with equal facility. There is no reciprocal relationship between cellular K and Na. *Tetrahymena* contains little Cl. Increases in cellular K or Na are not accompanied by increases in Cl.

3. The results are interpretable according to the following proposals: K is specifically accumulated and retained by a system of internal binding sites with a saturation level. Na is probably retained by a separate mechanism. There is also a Na extrusion mechanism which has no relationship with K or Na retention. Cellular K and Na are compartmentalized into three components: unexchangeable, exchangeable but bound, and freely diffusible components.

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